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EFFECT OF MONOMETHYLHYDRAZINE ON GLUCOSE LEVELS IN RATS

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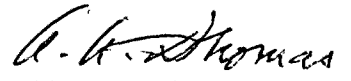

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Exposure to monomethylhydrazine (MMH) is known to affect glucose metabolism in rats although the exact mechanism is not known and reports in the literature reflect a wide disparity in experimental results. The variety of experimental conditions that were utilized could explain, at least in part, the differences in the results reported. Some of the factors that could affect glucose response to MMH were the mode of exposure, length of exposure, anesthesia used, the convulsigenic action of MMH, and the amount of liver glycogen stores prior to to exposure. The series of experiments reported here were performed to

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determine the influence of various experimental parameters on the glucose response to MMH exposure. Anesthesia was not required and all MMH exposures were below convulsigenic levels cancelling the effects of these two parameters. MMH was given in a single intraperitoneal injection and by intravenous infusion over a period of time. Both methods of exposure produced a hyperglycemic response but the response occurred at lower exposure levels when the MMH was continuously infused. The influence of the liver glycogen level prior to exposure on the glucose response was also evaluated. All groups of rats that had been either fed or fasted to deplete liver glycogen showed a similar hyperglycemia although the degree of response differed.

SUMMARY

Exposure to monomethylhydrazine (MMH) is known to affect glucose metabolism in rats although the exact mechanism is not known and reports in the literature reflect a wide disparity in experimental results. The variety of experimental conditions that were utilized could explain, at least in part, the differences in the results reported. Some of the factors that could affect glucose response to MMH were the mode of exposure, length of exposure, anesthesia used, the convulsigenic action of MMH, and the amount of liver glycogen stores prior to exposure. The series of experiments reported here were performed to determine the influence of various experimental parameters on the glucose response to MMH exposure. Anesthesia was not required and all MMH exposures were below convulsigenic levels cancelling the effects of these two parameters. MMH was given in a single intraperitoneal injection and by intravenous infusion over a period of time. Both methods of exposure produced a hyperglycemic response but the response occurred at lower exposure levels when the MMH was continuously infused. The influence of the liver glycogen level prior to exposure on the glucose response was also evaluated. All groups of rats that had been either fed or fasted to deplete liver glycogen showed a similar hyperglycemia although the degree of response differed.

PREFACE

This report represents research performed by the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from August 1974 to February 1976. The research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Procedures for Diagnosis and Treatment of Air Force Exposure Cases," Work Unit 63020215.

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INTRODUCTION

The use of monomethylhydrazine (MMH) in Air Force propulsion systems has prompted extensive investigations into its toxic properties and effects. One of the results of exposure to MMH is a derangement in glucose metabolism although reports are somewhat contradictory and inconclusive. Several investigators have reported marked hyperglycemia following MMH injection (O'Brien et al., 1964; Dost et al., 1971; Shawitz et al., 1972) but the results of other studies (Fortney and Clark, 1967) and previous unpublished work in this Laboratory have shown MMH causes an hypoglycemic response and depletion of liver glycogen. This dissimilarity in results can have several explanations: the level and duration of exposure, the mode of exposure, species differences, anesthesia effects, the relationship between MMH induced convulsigenic or preconvulsigenic activity and glucose levels, and the amount of glycogen storage before exposure. It was thought MMH might have an effect similar to hydrazine which causes an initial hyperglycemia and increased glycogenolysis followed by hypoglycemia. The degree and duration of hyperglycemia is directly proportional to the amount of liver glycogen available as a glucose source. In fasted animals with depleted glycogen stores there is no hyperglycemic phase and the hypoglycemic response to hydrazine is immediate (Fortney, 1966). However, when Dost (1973) infused glycogen depleted rats with MMH for three hours, the blood glucose levels rose sharply although not to the same degree as in rats that had adequate glycogen stores. Moreover, this rise in glucose began only a short time before the onset of convulsions. It is important to note that several of these studies on MMH induced hyperglycemia utilized high doses of MMH either approaching or at a convulsigenic threshold.

The experiments described here were done to determine the contribution and relationship of various experimental conditions to changes in glucose or glycogen levels following MMH exposure. As far as possible experimental conditions were rigidly standardized in an attempt to assess the effect of each variable separately. The amount of MMH that would produce a glucose response without convulsigenic activity was determined and this amount given by single intraperitoneal (I.P.) injection or by continuous infusion in all experiments. No anesthesia was required or used during exposures. Food intake was carefully controlled prior to exposure to assess the relationship of liver glycogen and plasma glucose levels after MMH injection. Groups of rats were fasted, fed ad lib, or given exogenous glucose to provide a range of plasma glucose and liver glycogen values prior to MMH injection.

METHODS AND RESULTS

Male Sprague Dawley rats, CFE strain, weighing between 350-425 grams were used in all experiments. Forty-eight or seventy-two hours before the experiment, polyvinyl cannulas were surgically implanted in rats that were to receive MMH or glucose by continuous infusion. For MMH infusion, the cannula was inserted into the carotid artery. One end was passed subcutaneously to a point just ahead of the withers, brought through the skin, filled with heparin

and capped. For glucose infusion the cannula was inserted into the duodenum, sutured to the wall and one end passed subcutaneously to the same area ahead of the withers, brought through the skin and capped. All rats were killed by cervical dislocation. Heparinized blood samples were taken from the heart, centrifuged and plasma glucose determined by the hexokinase method (Slein, 1963). The liver was immediately excised, rinsed with saline, blotted and frozen in liquid nitrogen until analyzed for glycogen. The tissue was weighed, homogenized in cold 10% trichloracetic acid and glycogen measured using the method described by Pfleiderer (1963). In some groups of rats, blood for glucose analysis was withdrawn at intervals from the carotid cannula.

Subconvulsive Level of MMH

The dose of MMH which would affect plasma glucose levels without causing convulsions was determined by injecting rats with various amounts of MMH. Ten groups of rats, four per group, were used; half were fasted 24 hours and half allowed food ad lib. MMH was injected I.P. in doses of 8, 12, 14, 16, or 22 milligrams MMH per kilogram body weight (mg/kg). Control rats were injected with saline. Plasma glucose levels were measured two hours following MMH injection. The results, as shown in Figure 1, indicate that injection

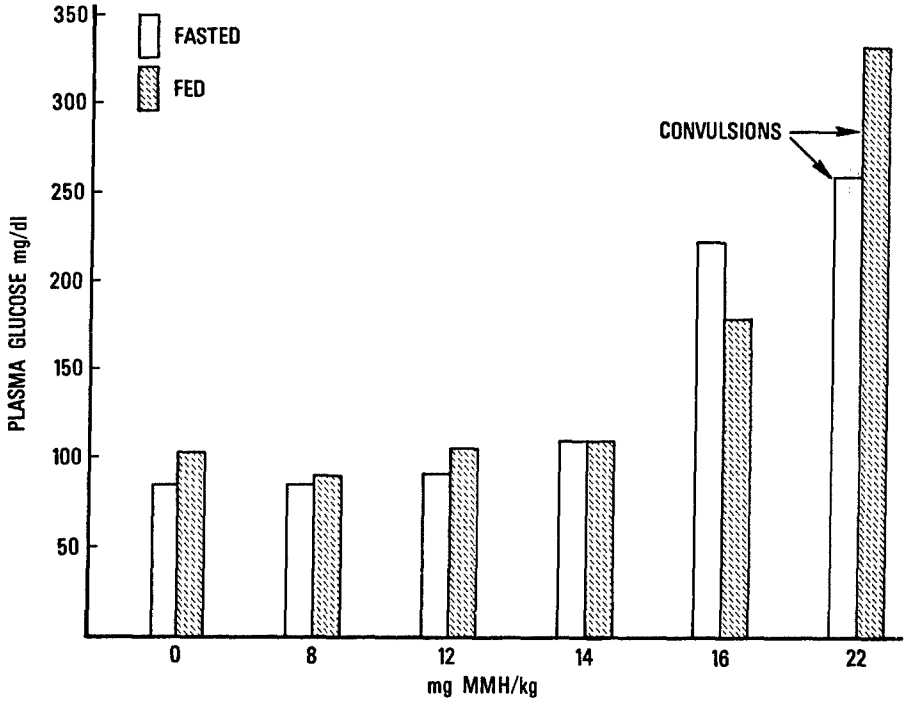

Figure 1. Plasma glucose levels in response to increasing amounts of MMH in fasting and fed rats.

of 8, 12, or 14 mg/kg did not produce a hyperglycemic response. Injection of 16 or 22 mg/kg did cause a rise in plasma glucose but at 22 mg/kg three of the four rats convulsed in both fed and fasted groups. There was no significant difference in response between fasted and fed rats. Therefore, 16 mg/kg was used as the dose level in all ensuing experiments.

Relationship of Liver Glycogen and Plasma Glucose Levels Following MMH.

The effect of MMH on plasma glucose and liver glycogen levels and the relationship of the pre-exposure glycogen level to the glucose response was determined. Rats were divided into groups of six rats; half were fasted 24 hours and half fed ad lib. In each group, four of the six rats were injected with 16 mg/kg MMH and the other two with saline. The groups of rats were killed and plasma glucose and liver glycogen determinations run at 0, 1, 2, 3, 4, 5, and 6 hours after MMH injection.

In addition, one group of fasted rats was sacrificed and the same measurements made nine hours following MMH. The results, Figure 2, show that plasma

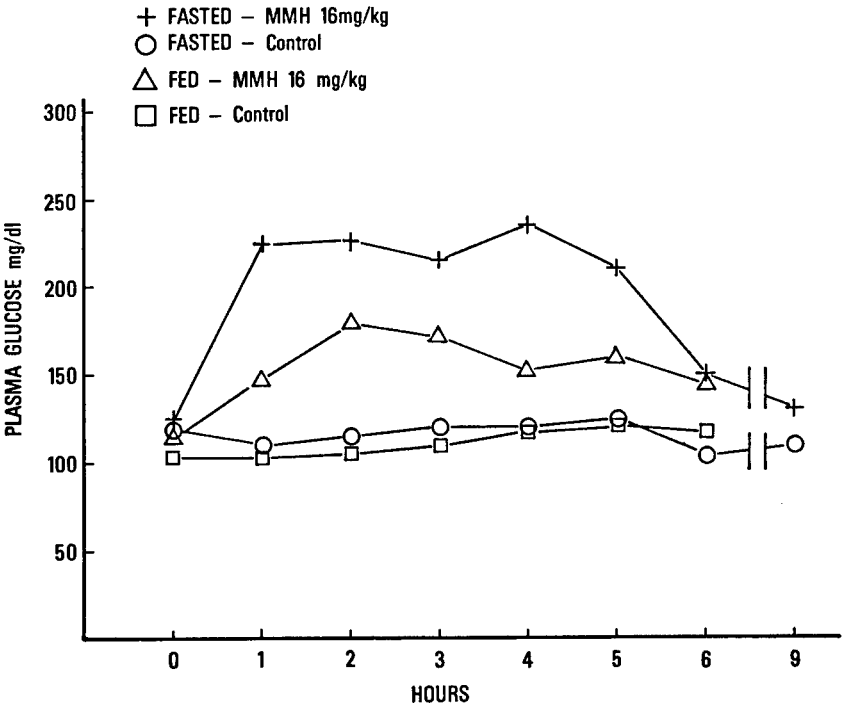

Figure 2. Plasma glucose levels in fed and fasted rats injected I.P. with 16 mg MMH/kg.

glucose levels in both fed and fasted rats were elevated within one hour after MMH and remained elevated about five hours. The values returned to baseline by nine hours. The fasted rats, both MMH injected and controls, had very low liver glycogen levels initially and there was no significant difference in the values of the two groups as shown in Table 1. The rats fed ad lib showed a wide individual range in glycogen levels and no significant difference could be noted between control and MMH injected rats. With the small number of animals killed at each time period it is possible the wide individual differences in the glycogen level masked any measurable effects of MMH.

TABLE 1

LIVER GLYCOGEN LEVELS IN FED AND FASTED RATS INJECTED WITH MMH

Glycogen mg/g Wet Weight*

	Fed		Fasted	
	MMH 16 mg/kg I.P.	Saline I.P.	MMH 16 mg/kg I.P.	Saline I.P.
Baseline	21.6 ± 1.3	26.8 ± 2.9	0.1 ± 0.1	0.9 ± 0.8
1 hour	25.9 ± 3.8	39.8 ± 7.5	0.3 ± 0.1	0.5 ± 0.2
2 hour	25.5 ± 2.1	25.2 ± 1.1	1.2 ± 0.1	0.8 ± 0.4
3 hour	30.6 ± 6.1	16.2 ± 1.4	0.3 ± 0.2	0.4 ± 0.3
4 hour	32.9 ± 6.3	13.8 ± 2.1	1.1 ± 0.4	0.4 ± 0.3
5 hour	18.8 ± 1.4	19.9 ± 1.9	1.4 ± 0.7	1.2 ± 0.6
6 hour	21.6 ± 6.5	40.8 ± 8.6	1.4 ± 0.4	0.1 ± 0.1

* Mean S.E. Mean

Effect of a Single Injection of MMH Compared to a Constant Infusion

This study was done to determine if infusion of 16 mg/kg MMH over five hours would produce a similar hyperglycemic response to that seen after a single injection and if the response occurred at the same concentration. Forty-eight hours prior to the experiment cannulas were placed in the carotid arteries of three groups of rats, six rats per group. The rats were fasted twenty-four hours and baseline plasma glucose levels run. One group was injected with 16 mg/kg MMH I.P.; one group of rats was infused with MMH at the rate of 3.2 mg/kg per hour in a volume of approximately 1 milliliter (ml) per hour for five hours; and, control rats were infused

with a similar volume of saline. Blood samples were taken at 1, 2, 3, 4, 5, and 6 hours after the start of the infusion for plasma glucose determinations. About 0.2 ml blood was taken at each period so the total blood loss was less than 1.5 ml. In the rats given MMH in a single I.P. injection the glucose level was elevated at one hour, peaked at four hours, and was almost at baseline by six hours. In the rats given 3.2 mg/kg over a five hour period the glucose level remained at baseline levels for the first three hours, began to rise between three and four hours, and was still elevated at six hours, one hour after MMH was discontinued. The hyperglycemic response in infused rats appeared when the total body burden was between 10 and 12 mg/kg. Glucose levels in control rats remained constant. This indicates the elevation in blood glucose occurs at lower concentrations of MMH when it is given as a constant infusion. When MMH was given as a single injection in doses of 8, 12, or 14 mg/kg, no hyperglycemic response was noted.

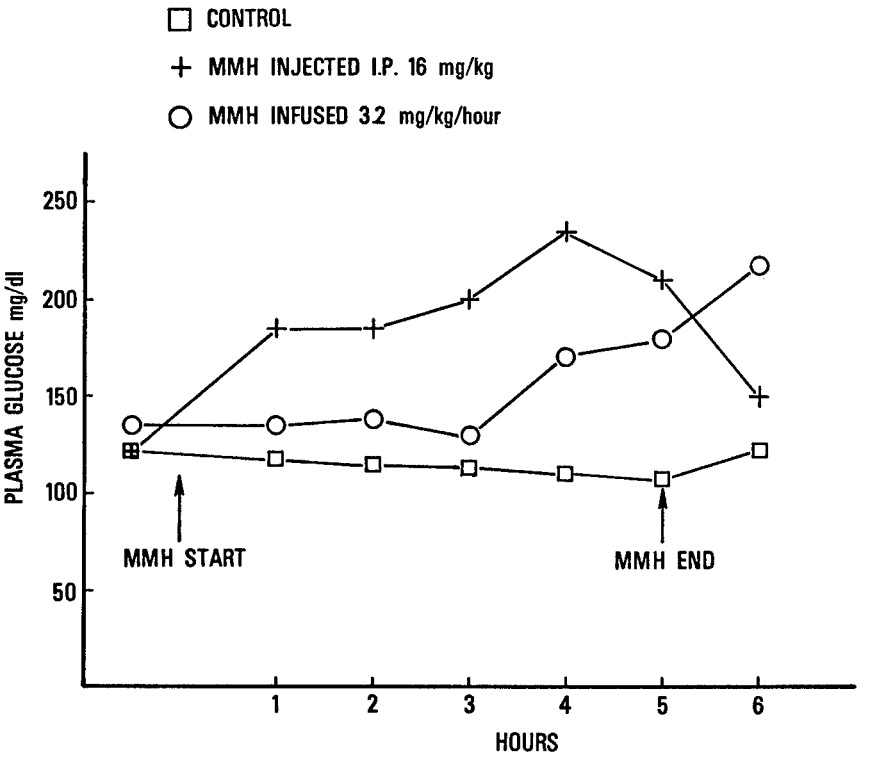

Figure 3. Plasma glucose levels in rats given 16 mg MMH/kg in a single I.P. injection or in an I.V. infusion for five hours.

Effects of MMH Infusion on Rats Receiving Exogenous Glucose

This experiment was done to determine the effect of MMH in rats receiving a constant supply of exogenous glucose at a rate calculated to provide a physiological level of glucose without causing glycogenesis. Two groups of rats, six rats per group, were surgically prepared with cannulas in the carotid arteries and in the duodenum. After a twenty-four hour fast, the rats were given 150 mg glucose per hour in a volume of about 1 ml per hour through the duodenal catheter. Previous trials had shown that this concentration of glucose would maintain a steady plasma glucose level of about 150 mg per decaliter (dl) without effecting an increase in liver glycogen. After one hour of glucose, a baseline plasma glucose level was determined and MMH infusion started at a rate of 3.2 mg/kg per hour for five hours in one group; saline was infused in the control group. Glucose was measured at 1, 2, 3, 4, 5, and 6 hour periods and glycogen levels measured at six hours. As shown in Figure 4, the hyperglycemic response again occurred between three to four hours after beginning MMH infusion and was still elevated at six hours. The glucose level was over 300 mg/dl indicating the hyperglycemia was more pronounced when exogenous glucose was supplied. The glycogen levels of the experimental and control rats were not significantly different.

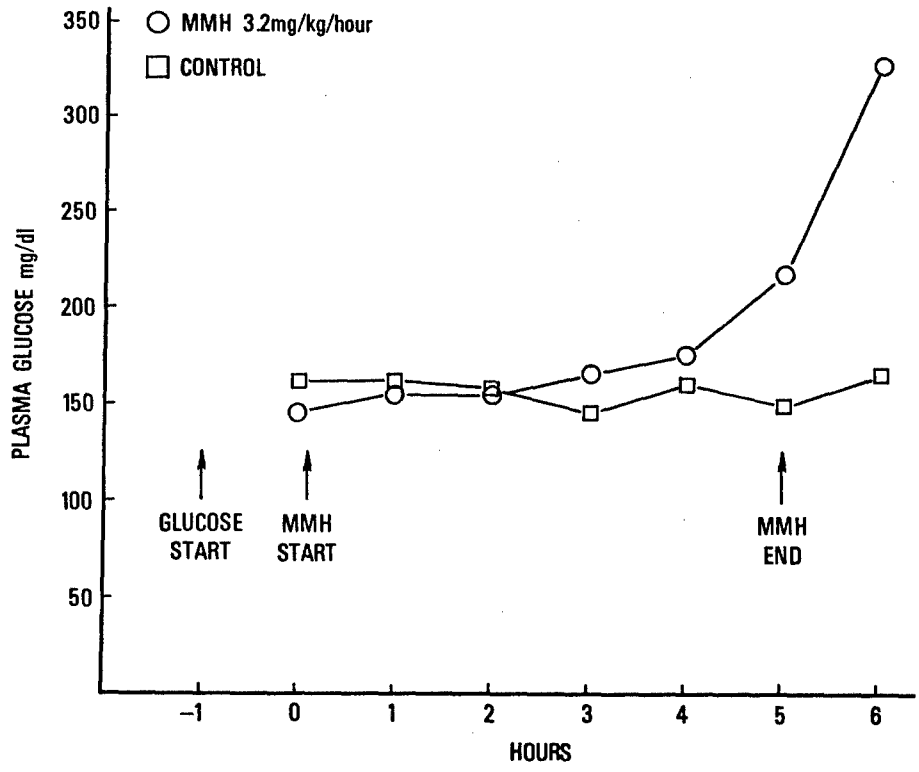

Figure 4. Plasma glucose levels in rats given 16 mg MMH/kg in an I.V. infusion for five hours and 150 mg glucose/hour by infusion into the duodenum.

DISCUSSION

The purpose of this study was to determine if MMH consistently produced a hyperglycemic response in rats at subconvulsive doses, the influence of the mode of exposure, the length of exposure and the relationship of the response to liver glycogen stores. We felt the conflicting reports in the literature could be partially explained by the differences in the experimental conditions. Our first goal was to establish the amount of MMH to be used that would elicit a positive result but would not be convulsigenic. Dost et al. (1973) had reported dramatic changes in blood glucose following infusion of 0.05 mM MMH/kg/hour for seven hours or 0.1 mM MMH/kg for three hours. In the seven hour test, there was a slight elevation in glucose about three hours after MMH was started with a sharp rise at seven hours. In the three hour test the glucose rose rapidly at about three hours. In each case the beginning of the sharp rise in glucose coincided with or immediately preceded the onset of convulsions which could have influenced the change in glucose levels. We found that a very sharply defined level of MMH, 16 mg/kg, given as a single I.P. injection was not convulsigenic but did cause hyperglycemia. This rise in glucose would seem to be a direct effect of MMH exposure rather than a response to convulsions.

We also observed that a lower concentration, between 10 and 12 mg/kg, when given intravenously as a continuous infusion, had the same hyperglycemic effect as a single dose of 16 mg/kg. However, when 12 mg/kg was given as a single injection there was no response. It had been noted previously that repeated doses of MMH produced toxic effects at a lower total dose than when given as a single dose. Dost et al. (1971) reported that multiple small doses cause convulsions or are lethal at a lower total dose than a single injection. They also found the production of $^{14}CO_2$ from labelled glucose was depressed at a lower dose of MMH if the MMH was infused continuously. Several factors could contribute to the increased response to lower total doses given by continuous exposure. In studies on the metabolic fate of MMH-^{14}C it was found that the percent converted to $^{14}CH_4$ and $^{14}CO_2$ increased as the dose decreased until at very low doses about 90% of the MMH given was converted (Dost et al., 1966). If the toxic effects of MMH are caused mainly by one or more of the metabolites produced in this conversion process, then multiple lower doses could be more efficient in converting a larger proportion of MMH to these toxic metabolites than a single higher dose. The continuous infusion of low levels of MMH would provide a constant supply of substrate for conversion to the effective compound if a metabolite is the principal toxicant. High doses may overwhelm the mechanism producing the metabolite or a large percent of the MMH may be excreted intact before being metabolized. It is excreted fairly rapidly with about 20-25% of the administered dose found in the urine within four hours (Pinkerton et al., 1967).

A major question in this study was the relationship between the liver glycogen levels and the hyperglycemic response to MMH. In fed rats there was no significant correlation between the degree of hyperglycemia and the amount of liver glycogen although the wide variation in glycogen levels between rats could mask any effect. In fasted rats with depleted liver

glycogen levels the elevation in blood glucose was still observed. In the rats given exogenous glucose the hyperglycemic response was more exaggerated than in the fasted rats or those allowed food ad lib. Muscle glycogen could be a source of glucose in the fasted rats although this would not account for the glucose levels seen. It is more likely that MMH causes an inhibition in glycolysis probably in the hexose portion of the anaerobic pathway prior to the triose step (Dost et al., 1973). One other possible mechanism that could cause hyperglycemia following MMH exposure would be an interference in the release of insulin. Since MMH is a monamine oxidase inhibitor, exposure to MMH could cause an intracellular accumulation of biogenic amines. Several reports have presented evidence that intracellular biogenic amines may inhibit insulin release (Aleyassine, 1971) and this could contribute to MMH produced hyperglycemia. This is currently under investigation in our Laboratory.

REFERENCES

1. Aleyassine, H. and S. H. Lee. "Inhibition by Hydrazine, Phenelzine, and Pargyline of Insulin Release from Rat Pancreas." Endocrinology 89:125-129, 1971.

2. Dost, F. N., D. E. Johnson, and C. H. Wang. "Effects of Monomethylhydrazine on Carbohydrate Metabolism." AMRL-TR-70-61, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, 1971. AD 727008

3. Dost, F. N., D. E. Johnson, and C. H. Wang. "Metabolic Effects of Monomethylhydrazine." AMRL-TR-73-33, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, 1973. AD 767596

4. Dost, F. N., D. J. Reed, and C. H. Wang. "The Metabolic Fate of Monomethylhydrazine and Unsymmetrical Dimethylhydrazine." Biochemical Pharmacology 15:1325, 1966.

5. Fortney, S. R. "Effects of Hydrazine on Liver Glycogen, Arterial Glucose, Lactate, Pyruvate, and Acid Base Balance in the Anesthetized Dog." Journal of Pharmacology and Experimental Therapeutics 153:562, 1966.

6. Fortney, S. R. and D. A. Clark. "Effect of Monomethylhydrazine on Methemoglobin Production In Vitro and In Vivo." Aerospace Medicine 38: 239, 1967.

7. O'Brien, R. D., M. Kirkpatrick, P. S. Miller. "Poisoning of the Rat by Hydrazine and Alkylhydrazines." Toxicology and Applied Pharmacology 6: 371, 1964.

8. Pfleiderer, G. "Glycogen Determination as D Glucose with Hexokinase, Pyruvate Kinase, and Lactic Dehydrogenase." Methods of Enzymatic Analysis, Ed. Hans-Ulrich Bergmeyer, Academic Press, New York, 1963.

9. Pinkerton, M. K., E. A. Hagan, and K. C. Back. "Distribution and

Excretion of ^{14}C-Monomethylhydrazine." AMRL-TR-67-175, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, 1967. AD 666662

10. Shaywitz, B. A., W. T. Gormley, M. E. George, and K. C. Back. "Effects of Monomethylhydrazine on Blood and Cerebrospinal Fluid Glucose in Anesthetized Monkeys." AMRL-TR-71-122, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, 1972. AD 751232

11. Slein, M. W. "Glucose Determination with Hexokinase and Glucose 6 Phosphate Dehydrogenase." Methods of Enzymatic Analysis, Ed. Hans-Ulrich Bergmeyer, Academic Press, New York, 1963.

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